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The paradigm shift in treatment from Covid-19 to oncology with mRNA vaccines

Jiao Wei, Ai-Min Hui [*]

Shanghai Fosun Pharmaceutical Industrial Development, Co., Ltd., 1289 Yishan Road, Shanghai 200233, China
Fosun Pharma USA Inc, 91 Hartwell Avenue, Suite 305, Lexington, MA 02421, USA

ABSTRACT

mRNA vaccines have gained popularity over the last decade as a versatile tool for developing novel therapeutics. The recent success of coronavirus disease (COVID-19) mRNA vaccine has unlocked the potential of mRNA technology as a powerful therapeutic platform. In this review, we apprise the literature on the various types of cancer vaccines, the novel platforms available for delivery of the vaccines, the recent progress in the RNA-based therapies and the evolving role of mRNA vaccines for various cancer indications, along with a future strategy to treat the patients. Literature reveals that despite multifaceted challenges in the development of mRNA vaccines, the promising and durable efficacy of the RNA in pre-clinical and clinical studies deserves consideration. The introduction of mRNA-transfected DC vaccine is an approach that has gained interest for cancer vaccine development due to its ability to circumvent the necessity of DC isolation, ex vivo cultivation and re-infusion. The selection of appropriate antigen of interest remains one of the major challenges for cancer vaccine development. The rapid development and large-scale production of mRNA platform has enabled for the development of both personalized vaccines (mRNA 4157, mRNA 4650 and RO7198457) and tetravalent vaccines (BNT111 and mRNA-5671). In addition, mRNA vaccines combined with checkpoint modulators and other novel medications that reverse immunosuppression show promise, however further research is needed to discover which combinations are most successful and the best dosing schedule for each component. Each delivery route (intradermal, subcutaneous, intra tumoral, intranodal, intranasal, intravenous) has its own set of challenges to overcome, and these challenges will decide the best delivery method. In other words, while developing a vaccine design, the underlying motivation should be a reasonable combination of delivery route and format. Exploring various administration routes and delivery route systems has boosted the development of mRNA vaccines.

Introduction

Vaccinations play a vital role in reducing disease, disability, and mortality from a variety of infectious diseases [1]. The use of conventional vaccines such as live attenuated vaccines, inactivated pathogens, subunit vaccines or toxoid vaccines provides durable efficacy against various infectious diseases [2]. Nucleic acid vaccines mainly, plasmid DNA(pDNA) and messenger RNA (mRNA), came to existence in 1900s due to their innate ability to stimulate inoculation with live organism-based vaccines, notably for cell-mediated immune stimulation [3]. For several decades later, pDNA-based approaches dominated the field, since mRNA-based approach was considered unstable due to inefficient in-vivo delivery and excessive stimulation of inflammatory responses [4,5]. Eventually in late 2000s, a series of improvement in manufacture,

modification and stabilization of mRNA led to its recognition as a resourceful platform for developing novel therapy [4,5]. mRNA vaccines thus hold a lot of promise and confer several advantages over traditional vaccines.

The recent outbreak of SARS-CoV-2 and coronavirus disease (Covid-19) has demonstrated an urgent need of rapid vaccine development. Two mRNA vaccines, BNT162b2 (Pfizer-BioNTech) and mRNA-1273 (Moderna TX), have acquired authorization from FDA that are currently being used to prevent COVID-19 [6]. Both vaccines have good efficacy as demonstrated in the various phase III trials and real world studies [7–11]. Knowledge gained from these trials and versatile therapeutic potential of the mRNA can be applied for the development of vaccine for the infectious diseases and cancer. In this review, we focus on the therapeutic aspect of mRNA vaccines as a cancer therapy. In

addition, we would apprise the literature on the various types of cancer vaccines, the novel platforms available for delivery of the vaccines, the recent progress in the RNA-based therapies and the evolving role of mRNA vaccines for various cancer indications, the available clinical and preclinical studies with the future chapter in treatment of patients.

The available platforms for development of anti-cancer vaccines

Cancer vaccines are a promising new immunotherapeutic strategy for both prevention and treatment. Vaccines targeting tumor associated or tumor-specific antigens (TAAs or TSAs) can destroy malignant cells that overexpress the antigens due to immunologic memory, resulting in a durable therapeutic response. Compared to other immunotherapies, cancer vaccines provide a precise, safe, and acceptable treatment. Currently, 2 prophylactic vaccines have been approved by the U.S. Food and Drug Administration (FDA) for routine use in clinical practice. Gardasil-9 is approved for prevention of HPV infection that is the cause of most HPV cancers. The other one is hepatitis B (HBV) vaccine, for example HEPLISAV-B, to prevent HBV infection that is known to cause hepatocellular carcinoma [12,13].

In 2010, PROVENGE (sipuleucel-T), an immune-cell based therapeutic cancer vaccine was granted approval for the treatment of individuals with asymptomatic or mild symptomatic metastatic castration-resistant prostate cancer (mCRPC) [14]. Besides, therapeutic vaccines are available for the treatment of early-stage bladder cancer (TheraCys® and TICE® Bacillus Calmette-Guerin (BCG)) [15] and melanoma [IMLYGIC® (talimogene laherparepvec/T-VEC)] [16]. Despite significant attempts to produce cancer vaccines, clinical translation of cancer vaccines into effective therapeutics has remained difficult for decades due to the wide range of tumor antigens and low immune response [17], originating the need to develop more potent vaccine approaches. Furthermore, there is a growing demand for vaccine development, large-scale manufacture, and dissemination, particularly in the case of non-viral diseases such as cancer [18,19].

In general, cancer vaccine platforms are classified into tumor cell, peptide, viral vector, dendritic cell (DC), DNA and RNA types (Fig. 1). Allogenic or autologous patient-derived tumor cells are used to make cellular vaccines [20]. This approach is beneficial in that target antigens does not have to be determined in advance [21]. The whole cell cancer vaccine approach using granulocyte–macrophage colony-stimulating factor (GM-CSF) has been studied in several types of cancer both in animals as well as human trials. The phase I and II studies with allogeneic GM-CSF–transduced vaccine post-radiation (derived from two pancreatic tumor lines) demonstrated durable efficacy and prolonged
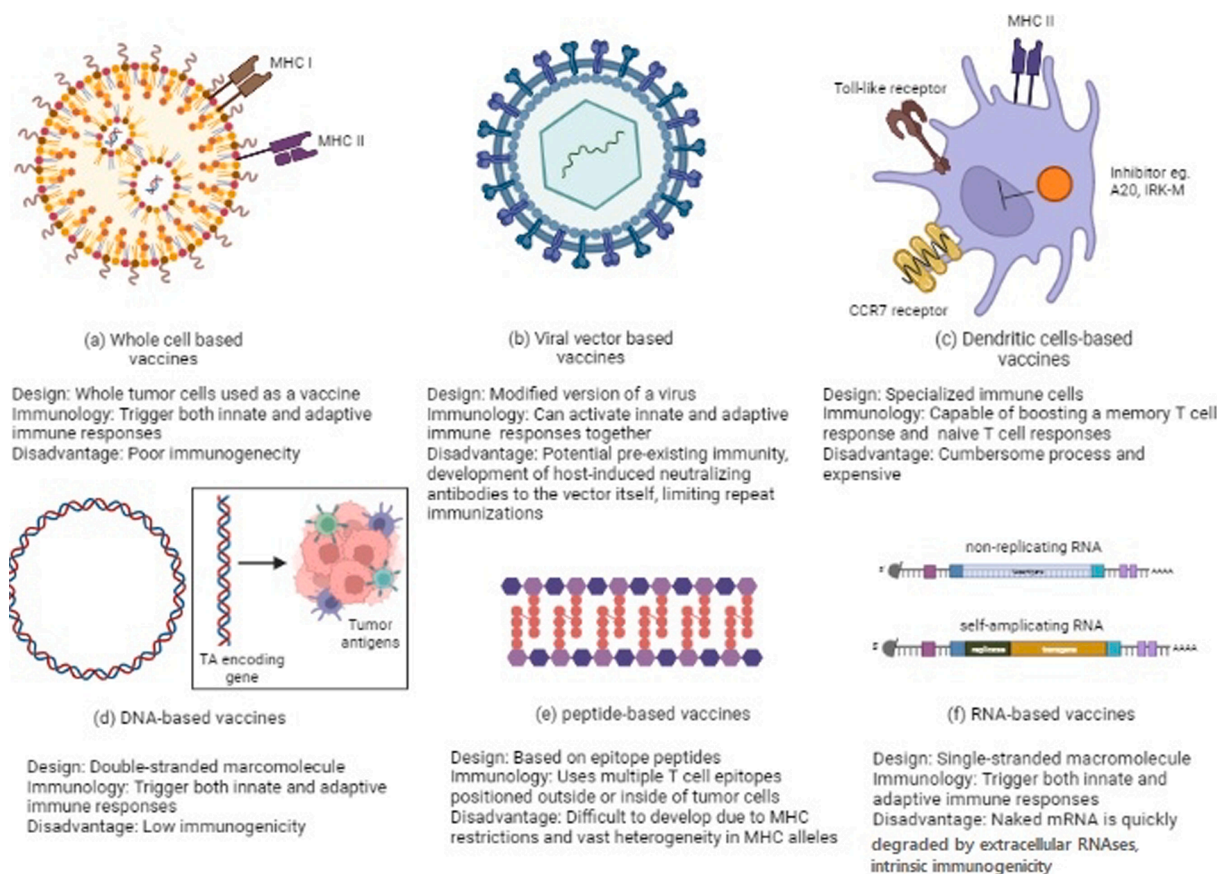

Fig. 1. The commonly available platforms and mechanisms for cancer vaccine development. (a) Whole cell-based vaccines (an autologous tumor cell vaccine using a patient's own cancer cells is injected as vaccine). (b) Viral vector-based vaccines (the genome of viral particles is modified to contain one or more genes encoding for the antigens of interest). (c) Dendritic cell-based vaccines (the dendritic cells efficiently capture the antigens, internalize, and process into peptides that are then presented in the context of MHC I and II molecules. These complexes are later recognized by the T-cell receptor (TCR) of CD8+ and CD4 + T cells) (d) DNA based vaccines (DNA plasmids are designed to deliver genes encoding TAs, eliciting or augmenting the adaptive immune response towards TA-bearing tumor cells. It induces the innate immune response, stimulates several DNA-sensing pathways in the cytosol of transfected cells due to the presence of CpG motifs and the double stranded structure itself) (e) Peptide-based vaccines (the peptides bind with the restricted MHC molecule expressed in APC. The peptide/MHC complex is then transported to the cell surface after intracellular processing and later recognized by the TCR on the surface of T cells, leading to activation of T lymphocytes) (f) RNA based vaccines (conventional non-replicating mRNA consists of 5 structural elements such as cap structures, a 5′ untranslated region (5′-UTR), an open reading frame encoding antigens of interest, a 3′-UTR; and an adenine repeating nucleotide sequence that forms a polyadenine (poly(A) tail. The non-replicating mRNA encodes antigen of interest, while self-amplifying mRNA encodes antigen of interest and a replication machinery, a self-replicating single-stranded RNA virus).

survival in patients with pancreatic cancer [22,23].

Peptide vaccines are made up of amino acid sequences that contain an epitope which can cause an immune response. Due to the difficulties of small peptides to attach directly to major histocompatibility complexes (MHC) I molecules, long peptides (containing of between 25 and 35 amino acids) are frequently favored over short peptides (consisting of approximately 10 amino acids). Short peptides also fail to activate CD4 helper T cells, which are required for full cytotoxic T lymphocyte activation (CTLs). These shortcomings can be overcome by using a long-peptide vaccine, that forces dendritic cells (DCs) to phagocytose the long-peptide before it is exposed on MHC I and attached to T cells. Long peptide vaccines also increase the HLA-related compatibility that exist with short-peptide vaccine. Furthermore, using a long peptide vaccine permits APCs to be presented via MHC II, which stimulates CD4+ lymphocytes, allowing for a more efficient immune response against tumor cells. However, because peptides are not self-immunogenic, administering an adjuvant at the same time is required for producing maximum efficiency [24]. So far, the peptide-based vaccines tested in laboratory has been able to elicit limited tumor-targeting immune responses, mostly because of intrinsic changes in cancer cells that reduce antigenicity and/or changes immunosuppressive alterations in the tumor microenvironment [25]. Therefore, other approaches are being developed including its combination with other immunotherapies, targeting antigenic epitopes arising from tumor cells and identifying target population [25].

Genetically modified viruses are also used for mRNA delivery. Application of positive strand RNA viruses via translation with host ribosomal machinery. However, challenges with host genome integration and the likelihood of host rejection, as well as cytotoxicity and immunogenicity, remains the major challenges. The MHC allows cancer cells to create peptide antigens that are present on their membrane surface. T cell receptors (TCRs) on cytotoxic T lymphocytes (CTLs) identify these antigens, resulting in cancer cell lysis. The antiviral immune response neutralizes viral vectors, limiting the number of vaccines that can be given [21].

Finally, to boost the adaptive immune system against tumor antigens, DNA cancer vaccines are created from bacterial plasmids (naked DNA) expressing one or more tumor antigens. The capacity of DNA vaccines lies in its ability to combine many genes expressing numerous tumor-antigens to establish a precise and broader adaptive immune response at the same time. However, these vaccines are poorly immunogenic [24]. To improve the immunological response of DNA vaccines, researchers have looked into encoding xenogeneic versions of antigens, fusing antigens with compounds that activate T cells or trigger associative recognition, DNA vector priming followed by viral vector boosting, and immunomodulatory molecules [26]. In contrast, RNA cancer vaccines are superior to DNA vaccines. While RNA is more susceptible to RNase breakdown, this can be minimized through chemical changes and the insertion of modified nucleosides such as pseudo uridine. Furthermore, unlike DNA, which must overcome the second barrier, the nuclear membrane, to reach the nucleus, RNA just needs to enter the cytoplasm [21]. The encoded proteins are converted into peptides that are present on MHC I and II to excite CD8+ and CD4+ T cells, respectively, after RNA translation. The fundamental pharmacology of mRNA vaccines is presented in Fig. 2.

Given the importance of DCs in initiating adaptive immunity in vitro and in vivo through generating CTLs, mRNA-transfected DC vaccine is an approach gaining interest for cancer vaccine development [24]. DC-based mRNA cancer vaccines have shown promising effects in various phases of clinical trials. Boczkowski and colleagues in 1996 first demonstrated that electroporation of DCs with mRNA could elicit potent immune responses against tumor in mice [27]. Since then, several human trials with electroporation of DCs have been conducted [28,29]. Bulk mRNA isolated from autologous tumors is another method for pulsing DCs with tumor antigen-loaded mRNA [30,31]. Direct injection of mRNA can be used instead of DC vaccines since it eliminates the need for DC isolation, ex vivo cultivation, and re-infusion [32]. Directly injecting the mRNA into secondary lymphoid tissue aids in delivering antigen to APCs at the T cell activation site, circumventing the need for DC movement [33].

Unlocking the potential of mRNA cancer vaccines

The cancer vaccines have the ability to elicit immune response to
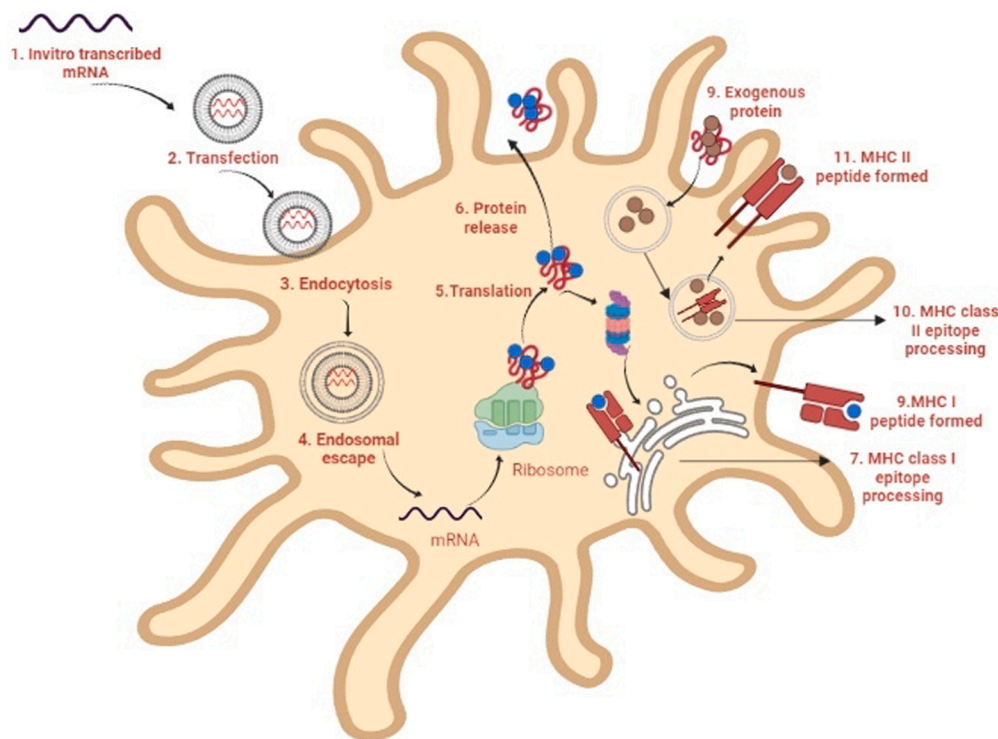

Fig. 2. Mechanism of action of mRNA vaccines. 1. In a cell-free system, mRNA is in vitro transcribed (IVT) from a DNA template. 2. IVT mRNA is then transfected into dendritic cells (DCs) by the process of (3) endocytosis. 4. Endosomal escape allows entrapped mRNA to be released into the cytoplasm. 5. The mRNA is translated into antigenic proteins using the ribosome translational mechanism. After post-translational modification, the translated antigenic protein is ready to act in the cell where it was produced. 6. The protein gets secreted by the host cell. 7. Antigen proteins are digested in the cytoplasm by the proteasome and transferred to the endoplasmic reticulum, where they are loaded onto MHC class I molecules (MHC I). 8. MHC I-peptide epitope complexes with loaded MHC I-peptide epitopes produced, resulting in induction. 9. Exogenous proteins are taken up DCs. 10. They are degraded in endosomes and delivered via the MHC II pathway. Furthermore, to obtain cognate T-cell help in antigen-presenting cells, the protein should be routed through the MHC II pathway. 11. The generated antigenic peptide epitopes are subsequently loaded onto MHC II molecules.

tumor antigens. The selection of a suitable target antigen is pivotal in the development of a vaccine design. Currently, the majority of vaccinations are TAAs, which are self-proteins that are improperly expressed by cancerous cells [21]. Developing vaccines against TAAs is challenging, as B- and T-cells might be subjected to removal by central and peripheral tolerance [34]. Besides, along with overexpression on tumor cells, TAAs might also be expressed in normal healthy cells leading to collateral damage [21]. In contrast, TSAs, which consists of neoantigens and viral oncoproteins are expressed only in cancerous cells. The prophylactic viral oncoproteins work by inducing the production of powerful neutralizing antibodies that block viral entrance into host cells and neoplasia caused by viruses [21]. However, these vaccines were ineffective in curing cancer as humoral immunity cannot effectively eliminate larger number of virus-infected cancer cells [21]. Neoantigens, like viral oncoproteins, are specific to tumor cells and are recognized by the immune system as foreign substances. Lately, neoantigens are being considered as a potential target in the progress of anti-cancer vaccine development. Numerous pre-clinical trials and early phase clinical trials have shown the ability of neoantigen based vaccines to minimize the potential induction of central and peripheral tolerance as well as the risk of autoimmunity [35,36].

TAAs with shared expression across cancer types, such as melanoma-associated antigen (MAGE1) and NY-ESO-1.37, has encouraged studies to target TAAs that are habitually overexpressed in a certain type of cancer, along with the prospect of generating a common vaccine per tumor type [37]. Empirical clinical experience has also suggested that vaccines targeting specific tumor antigens are ineffective in tackling tumor heterogeneity, as well as in dealing with the challenges of clonal evolution and immune evasion by the tumor [38]. As a result, with the increasing importance of therapeutic cancer vaccines, the rapid development and large-scale production using mRNA platform introduces the potential for the development of both personalized vaccines and off-shelf cocktail vaccines.

Personalized cancer vaccines (PCV)

The neoantigens remain unique for each individual, with their numbers varying on the type of cancer. This necessitates for a tailored approach in which the tumor genome is sequenced and mutations are detected, neoantigens are predicted using computerized algorithms and a vaccine is then created and delivered to the patient. Mice vaccinated with a computationally engineered synthetic mRNA comprising numerous MHC class II neoepitopes showed 100% tumor rejection in preclinical studies, demonstrating antigen distribution [39]. The safety and efficacy of this approach was established in a first-in-human clinical study involving 13 patients with metastatic melanoma. Each patient was given a vaccine that contained 10 neoepitopes specific to their tumor. In certain patients, antitumor responses were discovered in metastases removed after immunization, where T-cell infiltration and neoepitope-specific apoptosis of autologous tumor cells were discovered after vaccination. All patients exhibited CD4+ and CD8+ T-cell responses [40]. Since then, therapeutic cancer treatment with tailored mRNA vaccines has received a lot of interest, and several clinical trials are presently underway, according to the US National Library of Medicine. A recent study with mRNA-4650, a KRAS personalized vaccine, developed by Moderna and Merck, in combination with or without pembrolizumab was conducted to treat patients with pancreatic carcinoma. The lipid nanoparticles (LNPs) approach for delivery of mRNA-4650 showed well-tolerated anti-tumoral immune response [41]. Another personalized vaccine, mRNA-4157, targeting 20 TAAs and useful in treating various types of tumors, in single or in combination with pembrolizumab demonstrated acceptable safety profile with cytotoxic T-lymphocyte (CTL)- and memory T-cell-dependent immune responses [42]. Based on the ability of mRNA-4157 to elicit clinical response, a phase II trial is currently undergoing to evaluate the efficacy of the postoperative adjuvant therapy with mRNA-4157 and pembrolizumab in comparison

with pembrolizumab monotherapy in high-risk recurrent individuals with complete resection of tumor (NCT03897881). A first-in-human phase Ib study of RO7198457, a combination of systemically administered RNA-Lipoplex iNeST with the PD-L1 antibody atezolizumab is presently conducted in patients with locally advanced or metastatic solid tumors. The preliminary results of this study suggest significant level of neoantigen immune-tumor response. A randomized phase II study of RO7198457 in first-line for patients with melanoma in combination with pembrolizumab is currently ongoing, and 2 randomized clinical trials are planned for the adjuvant treatment of individuals with non-small cell lung cancer (NSCLC) and colorectal cancer (CRC) [43].

Tetravalent vaccine and combination therapies

A tetravalent RNA-lipoplex cancer vaccine, BNT111, contains 4 types of naked RNA such as RBL001.1, RBL002.2, RBL003.1, and RBL004.1 encoding 4 melanoma-associated antigens (MAAs), the cancer-testis antigen NY-ESO-1, the human MAGE- A3, tyrosinase, and putative tyrosine-protein phosphatase (TPTE), encapsulated in liposomes. The vaccine upon intravenous administration is taken up by the APCs, and after being translocated to the cytoplasm, is translated into the 4 tumor-associated proteins. As a result, CD8+ and CD4+ T-cell responses against 4 selected antigens are produced [44]. A phase I trial showed that this vaccine alone and in combination with immune checkpoint inhibitors (ICIs) induced durable objective responses and exhibited a favorable safety profile among patients with advanced melanoma [45]. A phase II trial is ongoing to evaluate the vaccine candidate in combination with the anti-PD-1 antibody cemiplimab for patients with unresectable stage III or stage IV melanoma who are refractory to or relapsed after anti-PD-1 therapy [46].

mRNA-5671, another tetravalent vaccine, is an LNP-formulated mRNA-based vaccine that targets 4 of the most frequent KRAS mutations (G12D, G13D, G12C and G12V). APCs take up and translate mRNA-5671 after immunization. Following translation, the MHCs displays the epitopes on the surface of APCs, resulting in the development of both cytotoxic T-lymphocyte- and memory T-cell-dependent immune responses directed at tumor cells with KRAS mutations [47]. CD8 T cell responses to KRAS antigens were considerably improved in preclinical investigations after immunization with mRNA encoding KRAS mutations [48]. Patients with advanced or metastatic NSCLC, colorectal cancer, or pancreatic adenocarcinoma and KRAS mutations are being enrolled in a phase I research using mRNA-5671 with or without pembrolizumab (NCT03948763).

Due to heterogenous and ever evolving nature of cancer mechanisms, the clinical benefit of monotherapy regimen in patients with advanced cancer is not adequate. Tumor-specific T lymphocytes produced by vaccines do not operate efficiently against the tumor due to their lack of motility and/or gradual depletion. As a result, combining procedure that prevent immune escape pathways is critical [49]. For instance, a phase II clinical trial in chemotherapy treated patients with metastatic castration-resistant prostate cancer (mCRPC) showed similar and durable tumor immune responses on addition of DC vaccines [50]. Monoclonal antibodies (mAbs) targeting CTLA-4 and the PD-1/PD-L1 expression have revolutionized the treatment paradigm for several types of cancers, including renal cancer, melanoma, bladder cancer, lung cancer and Hodgkin's lymphoma [51]. CureVac GmbH systemic mRNA immunotherapy and local irradiation therapy can eradicate established macroscopic E.G7-OVA and LLC cancers in a synergistic manner. Moreover, this combination boosted CD4+, NKT and CD8+ cell infiltration in tumor infected mouse [52]. CV9202, vaccine encoding 6 NSCLC-associated antigens (NY-ESO-1, MUC-1, MAGE-C2, MAGE-C1, 5T4 and survivin) have been proven to induce targeted immune responses. The combination of this vaccine with radiotherapy in a phase Ib clinical trial in 26 stage IV NSCLC patients revealed elevated CV9202 antigen-specific immune responses in 84% of patients, with 80% increased antigen-specific antibody levels, 40% patients with functional T cells

and about 52% of patients had multiple antigen specificities [53]. In another study, researchers used an mRNA vaccine expressing the TAA MUC1 in combination with an anti-CTLA-4 monoclonal antibody to boost the vaccine's immune response against triple-negative breast cancer (TNBC) by improving T cell activity [54].

Recent advancement of mRNA vaccines in various types of cancer

Preclinical and clinical evidence have shown that using mRNA for prophylaxis and therapy can help prevent infectious disease and treat cancers, and that mRNA vaccines are safe and well tolerated in both animal models and humans. Further enhancements might also boost antigen-specific immune responses as well as B and T cells immune responses [55]. As of 21st December 2021, 23 RNA vaccines are currently under phase I/II/III clinical trials, while 24 vaccines are at pre-clinical stage.

Breast cancer

Breast cancer remains a cause of mortality for women globally [56]. More often, 81% women suffer from invasive breast cancer, which comprises of at least 21 distinct histological subtypes and 4 molecular subgroups (luminal A, luminal B, triple-negative and HER2-enriched) that differ in risk factors, presentation, response to treatment, and outcomes [57]. Invasive breast cancer can spread to adjacent lymph nodes or other organs over time. It is because of widespread metastasis that a woman dies from breast cancer [58]. Using modern methodologies for mRNA sequencing, such as The Cancer Genome Atlas (TCGA) data, it has been established that increased expression of T- and B-cell predicts higher overall survival (OS) in a variety of tumor types, including breast cancer [59]. The current treatment approach for breast cancer includes radiation therapy, surgery, chemotherapy, as well as hormonal and targeted therapies. Lately, the development of medications that can prevent breast cancer from developing in the first place, as well as their recurrence, has gathered attention. The overexpression of high-affinity transmembrane receptors such as HER3, HER2, c-MET, EGFR, and the transmembrane protein epithelial mucin-1 (MUC-1) are the key oncogenic drivers for breast cancer [60]. Treatment of breast cancer, especially, TNBC is gaining importance, since lack of therapeutic targets makes such type of cancer unresponsive to typical endocrine therapies and HER2-targeted therapy. In such a case, cancer vaccines which aid in activation and amplification of TAA-specific immunity combined with a sustained memory T cell immune response may be an effective therapy for preventing breast cancer recurrence in patients [61]. Previous vaccination strategies in adjuvant settings, against HER2+ self-antigens have shown substantial efficacy in patients with breast cancer [62–64]. However, such an approach is usually weak as immune response as T-lymphocytes have affinity to HER2+ and thus are subject to central tolerance [65]. An ongoing phase I/II trial is being conducted in patients with TNBC and who completed standard of care chemotherapy, where patients are allocated to receive either 8 vaccination cycles of mRNA WAREHOUSE vaccine (containing pre-formulated, shared tumor antigens, non-mutated) or mRNA MUTANOME vaccine (containing individual mutations). The preliminary data of this trial showed mRNA WAREHOUSE is feasible approach for treatment of TNBC [66]. Another phase I trial from the Schmidt and colleagues was conducted with the addition of a third arm where patients were injected with IVAC_M_uID [Individualized NeoAntigen Specific Immunotherapy (iNeST)] which encodes 20 cancer mutations neoepitopes derived from NGS. The initial results reported promising results of iNeST IVAC_M_uID in inducing strong polyepitope T-cell responses in patients with TNBC in the post-(neo)adjuvant phase or post-surgery. All the patients reported CD4+ and/or CD8+ T-cell responses against 1 to 10 of the vaccine neoepitopes [67]. Theoretically, this treatment regimen will lead to a transition from an individualized therapy targeting a single biomarker (e.g., HER2) to a

fully specialized treatment targeting specific mutations in each patient. The ongoing trials related to mRNA vaccine in breast cancer is listed in Table 1.

Non-small cell lung cancer

Lung cancer remains a major cause of cancer worldwide after breast cancer. Despite recent therapeutic advancements, the overall 5-year survival rate for LC is still less than 20%. Because most cancers exhibit mutational variability, conventional cancer treatment techniques, such as surgery and chemotherapy, are far from optimum, especially for advanced stage malignancies. Currently, the information related to mRNA-based approach in treatment for NSCLC is limited. CV9201 is a cancer immunotherapy based on RNActive® that encodes 5 NSCLC antigens: melanoma antigen family C1/C2, NY esophageal squamous cell carcinoma-1, trophoblast glycoprotein and survivin. About 46 patients with locally advanced (n = 7) or metastatic (n = 39) NSCLC received 5 intradermal CV9201 injections (400–1600 g of mRNA) in a phase I/IIa dose-escalation experiment. After initial dose administration, the median progression-free survival and OS were 5.0 months (95 percent CI 1.8–6.3) and 10.8 months (8.1–16.7), respectively. In addition, 60% of patients reported an increased frequency of >2 fold followed by activation of IgD+ CD38hi B cells. This showed that CV9201 was well tolerated, and immunological responses could be observed following therapy, indicating that further clinical research is warranted [68]. The ongoing trials related to mRNA vaccine in breast cancer is listed in Table 2.

Prostate cancer

The standard treatment for prostate cancer includes androgen deprivation and chemotherapy. However, patients become resistant after prolonged treatment with these agents. Relapse or progression of disease occur even after complete androgen blockage and when plasma concentrations of testosterone are reduced to <50 ng/dL by castration or gonadotropin-releasing hormone analogs, and the effects of the remaining androgens are suppressed by androgen receptor antagonists [69]. With the advent of Sipuleucel-T, a dendritic-cell based vaccine, for treatment of advanced stages of prostate cancer, immunotherapy for prostate cancer has come into limelight. However, besides sipuleucel-T, there have been disappointing results in prostate cancer. In patients with mCRPC, large phase III studies of the CTLA-4 inhibitor, ipilimumab did not show significant benefit in OS compared to placebo before or after chemotherapy treatment. In addition, nivolumab, a single-agent PD-1 antibody, was found to have little effect in men with mCRPC. However, administering both CTLA-4 and PD-1 inhibitors combination has resulted in some PSA and objective responses, showing that a minority of patients may benefit. Pembrolizumab was given to mCRPC patients who were advancing on enzalutamide in a recent study, and a significant number of men had remarkable PSA and objective responses. PSA and objective responses appeared to be more common in another small trial

Table 1
Clinical trials for breast cancer.

Conditions	NCT number	Study design	Interventions	Status
Triple negative Breast Cancer	NCT02316457	Phase I	IVAC_W_bre1_uID/ IVAC_M_uID	Active, not recruiting
Breast Cancer	NCT00003432	Phase I/II	carcinoembryonic antigen RNA-pulsed DC cancer vaccine	Terminated
Breast Cancer	NCT03788083	Phase I	Trimix mRNA	Recruiting
Breast Cancer	NCT03739931	Phase I	mRNA-2752/ Durvalumab	Recruiting

Table 2
Clinical trials for non-small cell lung cancer.

Conditions	NCT Number	Study type	Interventions	Status
Non-Small Cell Lung Cancer	NCT03908671	Observational study	Personalized mRNA Tumor Vaccine	Not yet recruiting
Metastatic Non-small Cell Lung Cancer	NCT03164772	Phase II	Durvalumab/Tremelimumab/BI 1361849	Completed
Non-Small Cell Lung Cancer	NCT03948763	Phase I	V94/Pembrolizumab	Active, not recruiting
Non-Small Cell Lung Cancer	NCT04998474	Phase II	FRAME-001 personalized vaccine	Not yet recruiting
Non-Small-Cell Lung Cancer With Bone Metastases	NCT02688686	Phase I/II	Genetically modified dendritic cells + cytokine-induced killer	Unknown
Non-Small Cell Lung Cancer	NCT03166254	Phase 1	Pembrolizumab/NEO-PV-01 vaccine/Poly ICLC	Withdrawn
Stage IIIB/IV Non-Small Cell Lung Cancer	NCT00923312	Phase I/II	CV9201	Completed
Non-Small Cell Lung Cancer	NCT04355806	Prospective	PD-1/PD-L1 inhibitors/Inactivated trivalent influenza vaccine	Not yet recruiting
Stage II-III Non-Small Cell Lung Cancer	NCT04267237	Phase II	Atezolizumab/RO7198457	Withdrawn

combining ipilimumab and nivolumab than with either treatment alone, and it was suggested that patients with DNA repair gene mutations benefited the most. Finally, in a limited fraction of individuals with prostate cancer, pembrolizumab, which was recently licensed for mismatch repair-deficient or microsatellite-unstable tumors, might be beneficial. The use of synthetic nucleotide-based DNA or RNA vaccines is an alternative route for in vivo cancer vaccine design. The use of plasmid DNA expressing TAAs to stimulate humoral and cellular immune responses has been shown earlier. However, in contrast to the features of mRNA, the potential of DNA-based anti-cancer vaccines integrating into the host genome and resulting in malignant transformation is a major barrier. Due to the instability of natural mRNA molecules, Cure Vac (Tubingen, Germany) had developed RNActive® vaccines, which are mRNA molecules optimized to elicit powerful, well-balanced immunological responses including humoral and cellular responses, effector and memory responses, and Th1 and Th2 immune cell activation. These molecules stimulate the immune system by inter-reacting with toll-like receptor 7 and do not modify the primary amino acid sequences [70]. Initial assessment of immune response in compounds encoding prostate specific membrane antigen (PSMA) or oval albumin demonstrated strong humoral immune response with Th2 and Th1 cells, with repeated immunization increasing the frequency of IFN-γ-secreting CD8+ T cells while maintaining CD4+ regulatory T cells frequency [70].

Early intervention in patients with hormone-refractory prostate cancer with CV9103 elicited significant cytotoxic T-cell response against all tested PSAs. A phase I/IIa clinical study with CV9103, a prostate cancer vaccine containing 4 antigens, mainly, tumor associated antigens PSA, PSMA, prostate stem cell antigen and six transmembrane epithelial antigen of the prostate 1, displayed a high level of immunogenicity in patients with mCRPC, where, 58% responders reacted against multiple antigens. About 74% patients had antigen-unspecific B-cells, while 79% of responders had antigen-specific T-cells. One patient displayed >85% drop in his PSA-level [71]. Though the initial responses in these trials were encouraging, the subsequent trial with CV9104 for prostate cancer were terminated due to no significant effect on OS [72]. These findings indicate that selection of antigen is crucial for activating APCs and immune response. Several studies have highlighted the efficacy of mRNA vaccine in other therapeutic areas [73,74]; when MS2 delivery platform is used. Using recombinant protein technology, the MS2 capsid can interact with specific 19-nucleotide stem-loop, can pack the target RNA, thereby preventing degradation by nucleases [75]. Li et al. observed that MS2 virus-like particles (VLPs)-based hPAP–GM–CSF mRNA vaccine might decrease prostatic-tumor growth in C57BL/6 mice, implying that this vaccine could elicit an effective immune response in a short period of time and is a viable treatment for prostate tumors [76]. The recent advancement in prostate cancer is the delivery of mRNA as nanoparticle. The co-delivery of C16-R848 adjuvant-pulsed mRNA vaccination with OVA RNA increased TAA presentation while simultaneously stimulating CD8+ T cell expression into the tumor and improved the overall anti-

tumor response, demonstrating effective adaptive immune. The vaccine significantly reduced 80% of tumor growth when given before tumor engraftment and suppressed tumor growth by 60% when given post tumor engraftment in syngeneic allograft mouse models of lymphoma and prostate cancer. These data imply that adding C16-R848 adjuvant pulsation to mRNA vaccine NP is a rational design strategy for improving the efficacy of synthetic mRNA vaccines [77]. Further, clinical trials related to mRNA vaccine in prostate cancer is listed in

Table 3
Clinical trials for prostate cancer.

Study population	NCT number	Study design	Intervention	Status
Hormonal Refractory Prostate Cancer	NCT00831467	Phase I/II	CV9103	Completed
mCRPC	NCT01153113	Phase I/II	hTERT mRNA DC	Withdrawn
Prostate Cancer	NCT01197625	Phase I/II	Dendritic cell vaccine	Active, not recruiting
Prostate cancer	NCT01278914	Phase I/II	Dendritic Cells (DC) prostate	Completed
mCRPC	NCT01817738	Phase I/II	CV9104	Terminated
Prostate cancer	NCT01446731	Phase II	mRNA transfected dendritic cell/Docetaxel	Completed
Prostate Cancer	NCT00006430	Phase I	Autologous dendritic cells transfected with amplified tumor RNA	Unknown
Prostate Cancer	NCT02140138	Phase II	CV9104	Terminated
Prostate Cancer	NCT02692976	Phase II	mDC vaccine/pDC vaccine/mDC and pDC vaccine	Completed
Prostate cancer	NCT00108264	Phase I	Tumor RNA transfected dendritic cells	Completed
Prostate cancer	NCT00004211	Phase I/II	PSA RNA-pulsed dendritic cell vaccine	Completed
Prostate cancer	NCT00010127	Phase I	Therapeutic autologous dendritic cells	Terminated
Prostate cancer	NCT04382898	Phase I/II	BNT112 with Cemiplimab	Recruiting
Hormonal Refractory Prostate Cancer	NCT00906243	Phase I/II	CV9103	Terminated
Prostate cancer	NCT01784913	Phase I/II	UV1 synthetic peptide vaccine and GMCSF	Active, not recruiting

Table 3.

Lymphoma

Lymphomas are a biologically and clinically heterogeneous group of carcinomas that develop in secondary lymphoid organs from mature B- or T-lymphocytes [78]. Global statistics reveal Hodgkin lymphoma occurs in 0.4% of total cancer population, while, non-Hodgkin lymphoma (NHL) is more frequent and accounts for 2.8% of all types of cancer [56]. With the increase in the incidence of lymphoma and no known effective treatment, there is an urgent need to develop novel therapies [79]. Patients with lymphoma have been benefitted from monoclonal antibodies such as rituximab, however, majority of patients remain incurable or die of the disease [79]. The identification of B-cell receptor variable regions as B-NHL unique antigens aided the development of tailored made vaccines to protect patients against their own tumors. Despite promising early results, this technique has yet to demonstrate enough clinical value to gain regulatory approval [80]. Use of personalized and standardized approach have been tested earlier, but have experienced drawbacks, with slim chance of success in clinical trials. Further, tumor-induced immunosuppressive factors and immune regulatory mechanism might limit the ability of immune system to generate antitumor immune response. Currently, the mRNA vaccine approaches are mostly in the nascent stage with preclinical studies demonstrating promising efficacy in reducing tumor growth. As mentioned earlier in the paragraph for prostate cancer, the co-delivery of C16-R848 adjuvant-pulsed mRNA vaccination with OVA RNA significantly reduced tumor growth in syngeneic allograft mouse models of lymphoma [77]. Similarly, in another study, 6 female C57BL mice were subcutaneously injected with E.G7 OVA expressing lymphoma cells to test the therapeutic efficacy of mRNA Galsomes over NPs containing unmodified mRNA. After intravenous administration, mRNA Galsomes transmits nucleoside-modified antigen-encoding mRNA as well as the glycolipid antigen and immunopotentiator α-galactosyl ceramide to APCs. Both the treatments showed significant tumor reduction in 40% of animals and prolonged OS. Further combination of mRNA Galsomes with PD-L1 checkpoint inhibitor indicated a synergistic behavior in tumor reduction [81]. The clinical trials related to mRNA vaccine in lymphoma is listed in Table 4.

Pancreatic cancer

Pancreatic cancer is a fatal malignancy with survival rate of 10.8%

Table 4
Clinical trials for lymphoma.

Conditions	NCT Number	Phases	Interventions	Status
Primary/ Relapsed Acute Lymphoblastic Leukemia	NCT03559413	Phase I/II	Individual peptide vaccination with adjuvant GM-CSF and Imiquimod	Active, not recruiting
Acute Leukemia/ Acute Lymphoblastic Leukemia/ Acute Myeloid Leukemia	NCT04969601	Phase I/II	Vaccine COMIRNATYÂ® (BNT162b2)	Recruiting
Relapsed/ Refractory Solid Tumor Malignancies or Lymphoma	NCT03739931	Phase I	mRNA-2752/ Durvalumab	Recruiting
Relapsed/ Refractory Solid Tumor Malignancies or Lymphoma	NCT03323398	Phase I/II	mRNA-2752/ Durvalumab	Active, not recruiting
Lymphoma	NCT04847050	Phase II	mRNA-1273	Recruiting

over 5 years [82]. The pancreatic cancer cells are also distinguished by several germline or genetic mutations including KRAS (90%), TP53 (75%–90%), CDK2NA (90%), SMAD4/DPC4 (50%). Surgical resection is a possible treatment for this type of cancer, however, primarily, the cancer goes undetected at an earlier stage and those who opt for surgery show signs of recurrence within 2 years after operation. The other treatment strategies include combination chemotherapy, immune checkpoint inhibitors and targeted therapies. The aggressive nature of the tumor cells along with the hostile tumor microenvironment nature has resulted in the chemoresistance [83]. Hence, the target of recent clinical investigations have been shifted to newer therapies such as macrophage and cytotoxic T-lymphocyte targeted therapies, adoptive T-cell therapy and cancer vaccines [84]. Designing a pancreatic cancer vaccine based on peptide, tumor cell, dendritic cell or DNA based system has several disadvantages leading to poor therapeutic efficacy [85–88]. On the contrary, mRNA cannot incorporate into the genome and hence does not pose any risk of insertional mutagenesis, with a superior safety profile. Nonetheless, mRNA vaccine against pancreatic cancer antigens has remained underdeveloped so far, and no suitable patient population has been identified. A recent study by Huang et al, identified 6 potential antigens, namely, WNT7A, ADAM9, MET, EFNB2, TPX2 and TMOD3 for mRNA vaccine development [89]. Patients with immune subtypes 4 and 5 considered as immunological "cold" phenotypes were found to be suitable for vaccination [89]. A list of clinical trials for the development of pancreatic cancer vaccine is reported in Table 5.

Melanoma

Melanoma is a malignant tumor that originates from melanocytes, with a 5-year survival rate of 10% in patients with end-stage melanoma [90]. Nowadays, several therapies are available, including chemotherapy, radiation therapy, immunotherapy, and surgery. Of these, immunotherapy with ipilimumab, nivolumab and pembrolizumab have been approved as standard therapy for cutaneous melanoma. Chemotherapeutic treatment regimens damage the normally dividing cells along with tumor-infected cells [91]. Hence, the process of development of further treatment strategies which suppress tumor growth is being explored. mRNA based vaccines are the latest development for treatment of melanoma. An initial phase I/II trial in 21 metastatic melanoma patients co-injected with protamine-protected mRNA induced antitumor immune response. Especially in patients injected with keyhole limpet hemocyanin (KLH) along with vaccine, the frequency of Foxp3+/CD4+ regulatory T cells decreased upon mRNA vaccination in their peripheral blood, whereas myeloid suppressor cells (CD11b + HLA-DRlo monocytes) were reduced in the patients not receiving KLH [92]. A recent application of personalized RNA mutanomes in 5 humans demonstrated

Table 5
Clinical trials for pancreatic cancer.

Conditions	NCT Number	Study design	Interventions	Status
Pancreatic Cancer	NCT04157127	Phase I	Autologous DC vaccine	Recruiting
Pancreatic Cancer	NCT05116917	Phase II	Nivolumab/ Ipilimumab/Influenza vaccine/Stereotactic body radiation therapy	Recruiting
Pancreatic Cancer	NCT04627246	Phase I	Autologous Dendritic Cell Vaccine Loaded with Personalized Peptides (PEP-DC vaccine)	Recruiting
Pancreatic Cancer	NCT03948763	Phase I	V941/Pembrolizumab	Active, not recruiting
Pancreatic Cancer	NCT04161755	Phase I	Atezolizumab/ RO7198457/ mFOLFIRINOX	Active, not recruiting

prolonged progression-free survival. Two of the five patients experienced vaccine-related objective responses, while 1 patient had a late relapse suggesting acquired resistance mechanism. The third patient develop complete response to vaccination in combination with PD-1 blockade therapy [40]. Another preclinical study in C57BL/6 mouse model of B16F10 melanoma reported the promising immune response of lipid encapsulated mRNA vaccine encoding TRP2. In addition, co-delivery of mRNA vaccine and PD-L1 siRNA downregulated PD-L1 in the dendritic cells promoting T cell activation and proliferation, in turn inhibiting tumor growth and metastasis [93]. Various combinations of mRNA with ICIs are currently being explored in clinical trials. For instance, Wilgenhof et al. assessed the anti-tumor activity of TriMixDC-MEL (an autologous monocyte-derived dendritic cell electroporated with synthetic mRNA encoding CD40 ligand) in patients with pretreated advanced melanoma, either as a monotherapy (NCT01066390) or combined with ipilimumab (NCT01302496) and in disease free melanoma patients following local treatment of macro metastases. The median progression-free survival and overall survival was substantially improved in both the groups with more durable increase in patients with combination therapy [94]. An interim analysis by Sahin et al. showed that BNT111 alone or in combination with PD1 inhibitors, mediates durable objective responses in checkpoint-inhibitor experienced patients with unresectable melanoma. These responses were accompanied by strong induction of CD4+ and CD8 + T cell immunity against the vaccine antigens [45]. A list of clinical trials for the development of melanoma vaccine is reported in Table 6.

Several clinical investigations for mRNA vaccines in various type of cancer have reported promising preliminary results. A list of clinical trials along with their results are presented in Table 7.

Optimization of the mRNA vaccine pharmaceutical features

Vaccine design and modification

The development of cancer vaccine depends on type of cancer, vaccine design and its modification and route of delivery (Fig. 3). The mRNA vaccine is presented under 2 categories: self-amplifying RNA (saRNA) and nonreplicating mRNA. The typical non-replicating mRNA comprises of a cap, flanked by 5′-untranslated regions (UTR) and 3′-UTRs, open reading frame (ORF) encoding vaccine antigens and poly(A) tail. Similar to conventional mRNA vaccine, mRNA is prepared synthetically by in vitro transcription of a linearized plasmid DNA or PCR construct containing the targeted gene and a promoter region when bacteriophage polymerase binds and initiates synthesis [95]. While the saRNA is more complex and comprises of the coding sequences of a viral replicase complex, a genomic and a sub genomic promoter, along with the basic elements of a conventional mRNA molecule. The change of mRNA's non-coding regions (5′ cap structure and capping efficiency, 5′- and 3′ UTRs), 3′ poly(A) tail), and nucleoside base modifications are all part of the optimization process.

Codon optimization

Translation efficiency is known to be influenced by codon composition. The rate of protein production and the time spent in the ribosome repository can be affected by mRNA sequence codon optimization [96]. It was discovered that replacing a nucleotide with N1-methyl-pseudouridine (N1mΨ) improves base pair stability, resulting in a complex secondary structure and better mRNA translation [96]. Substituting rare codons with regular identical codons that contain plenty of similar tRNA in the cytosol is a common practice to alleviate mRNA production [97]. Although a high GC sequence may cause problems with mRNA secondary structure, it translates 100-fold higher than a low GC sequence [98].

Table 6
Clinical trials for melanoma.

Conditions	NCT Number	Study design	Interventions	Status
Melanoma	NCT02410733	Phase I	Tetravalent RNA-lipoplex cancer vaccine targeting 4 TAAs (RBL001.1, RBL002.2, RBL003.1, and RBL004.1	Active, not recruiting
Metastatic Melanoma	NCT00672542	Phase I	Proteasome siRNA and tumor antigen RNA-transfected dendritic cells	Completed
Melanoma	NCT01684241	Phase I	RBL001/RBL002	Completed
Melanoma	NCT04526899	Phase II	BNT111/ Cemiplimab	Recruiting
Melanoma	NCT00126685	Phase I/II	autologous tumor cell vaccine/ therapeutic autologous dendritic cells	Unknown
Melanoma	NCT01456104	Phase I	Langerhans-type dendritic cells	Active, not recruiting
Melanoma	NCT05264974	Phase I	Autologous total tumor mRNA loaded DOTAP liposome vaccine	Not yet recruiting
Melanoma	NCT02035956	Phase I	Ivac mutanome, rbl001/rbl002	Completed
Metastatic Melanoma	NCT01216436	Phase I	RNA-transfected mature autologous DC	Terminated
Melanoma	NCT00074230	Phase I/II	Autologous Dendritic Cells loaded with MAGE-A3, MelanA and Survivin	Completed
Melanoma	NCT01676779	Phase I/II	mRNA Electroporated Autologous Dendritic Cells	Completed
Resected melanoma	NCT03394937	Phase I	ECI-006	Terminated
Stage III/IV Malignant Melanoma	NCT01066390	Phase I	TriMixDC	Completed
Stage III/IV Malignant Melanoma	NCT01302496	Phase II	TriMix-DC and ipilimumab	Completed
Malignant Melanoma	NCT00204516	Phase I/II	mRNA coding for melanoma associated antigens	Completed
Advanced Malignant Melanoma	NCT01278940	Phase I/II	Dendritic Cells loaded RNA	Completed
Advanced Melanoma	NCT03815058	Phase II	RO719845/ Pembrolizumab	Active, not recruiting
High-Risk Melanoma	NCT03897881	Phase II	mRNA-4157/ pembrolizumab	Active, not recruiting
Melanoma	NCT02285413	Phase II	DC based mRNA/ cisplatin	Completed
Melanoma	NCT00204607	Phase I/II	mRNA	Completed
Metastatic melanoma	NCT00961844	Phase I/II	Tumor-derived mRNA/Temolomide	Terminated
Melanoma	NCT01530698	Phase I/II	Autologous dendritric mRNA	Completed
Melanoma Stage III or IV	NCT00243529	Phase I/II	Autologous dendritric mRNA	Completed
Breast Cancer or Malignant Melanoma	NCT00978913	Phase I	DC mRNA	Completed

Table 6 (*continued*)

Conditions	NCT Number	Study design	Interventions	Status
Melanoma	NCT00940004	Phase I/II	DC mRNA	Completed
Melanoma	NCT03480152	Phase I/II	NCI-4650	Terminated
Stage III/IV malignant melanoma	NCT01973322	Phase II	DC mRNA	Recruiting

Noncoding region optimization

The 5′ and 3′ UTR elements bordering the coding sequence have significant impact on the stability and translation of mRNA, both of which are crucial considerations in the optimal vaccine design. These optimization increases the efficiency and half-life of mRNA [99,100]. For effective mRNA protein synthesis, a 5′ cap structure is essential [101]. This can be achieved by applying 5′ cap in multiple versions during or after the transcription process by using a vaccinia virus capping enzyme [102] or by incorporating synthetic cap or anti-reverse cap analogues [103]. An appropriate length of poly(A) tail also plays a critical role in regulation of mRNA translation and stability, thus it must be inserted directly from the encoding DNA template or with poly(A) polymerase [104]. A recent study suggested that mRNAs with phosphorothioate groups within the poly(A) tail were less sensitive to 3′-deadenylase degradation than unmodified mRNA and were more efficiently produced in cultured cells, paving the way for future progress of mRNA-based therapeutics [105].

Modifications of untranslated regions of mRNA also represents one of the approaches to enhance both mRNA efficiency and stability. Warren et al. used a synthetic 5′ UTR with a strong Kozak translation signal and the alpha globin 3′UTR to increase protein synthesis during fibroblast conversion to induced pluripotent stem cells [106]. Elsewhere, the globin 3′UTR has been used to increase mRNA stability since globin mRNAs generate large amount of protein with longer half-life [99]. Recently, in a review article by Miao et al. suggested 3 steps for modification of UTR as: "avoid the presence of start codon (AUG), and non-canonical start codons (CUG) in the 5′ UTR, second, avoid the presence of highly stable secondary structures, which can prevent ribosome recruitment and codon recognition. Thirdly, shorter 5′UTR may be introduced as previous studies have shown that this type of 5′UTR is more conducive to mRNA translation process" [17]. A screening method using a diverse set of 5′UTR and 3′UTR combinations for better expression of the Arginase 1 protein highlighted 5′ UTR as an essential driver in protein expression for exogenously delivered mRNA [107].

Elimination of pathogen-associated molecular patterns in mRNA via incorporation of modified nucleosides, such as pseudouridine [108] and 1-methylpseudouridine (m1Ψ) [109], and fast protein liquid chromatography purification to remove double-stranded RNA contaminants [110] is another approach to improve mRNA therapeutic efficiency. An advantage of such optimization is that the vaccine is able to bypass the transcription process directly starting the translation phase to produce the immunogenic protein inside the human cells [33].

Self-amplification vaccine

Self-amplifying mRNA (SAM) vaccines are derived from an α-virus genome, which enables the intact RNA replication but the structural protein genes substituted with the antigen of interest. Due to intracellular replication of the antigen-encoding RNA, the SAM can produce a significant amount of antigen from a very little vaccination dose [33]. SAM vaccines are capable of creating their own complements of dsRNA structures, replicate intermediates and other features which could contribute to their high effectiveness. However, due to the inherent nature of these RNAs, modulating the inflammatory profile or

reactogenicity of SAM vaccines may be difficult [33]. The applications of SAM in cancer vaccine development are however limited to animal models, and 2 clinical trials against colorectal cancers (NCT01890213 and NCT00529984).

Delivery format

Due to negatively charged structure of naked RNA and large molecular size, mRNA is prone to nuclease degradation and cannot cross the cell membrane. Thus to overcome this obstacle, several mRNA vaccine delivery strategies have been employed such as, naked mRNA delivery, mRNA delivery through viral vectors, polymer-based vectors, lipid-based vectors, lipid-polymer hybrid nanoparticles, and peptide-based vectors [4,111], Subcutaneous administration has been found very efficient for translation of encoded protein for mRNA, with the ability to induce both cellular and immune response through this route. However, the outermost layer of skin represents a tough barrier for drugs absorption and hence various approaches have been adopted to overcome this barrier, including microneedles, microporation, and jet injection, electroporation, iontophoresis, sonophoresis, formulation as NPs and liposomes [112].

Lipid-based vectors/nanoparticles

The LNPs are derived from cationic lipids containing tertiary or quaternary amines to encapsulate polyanionic mRNA. A study reported antigen-specific CTL activity and suppressed the OVA-suppressing tumors in mice injected with OVA-encoding mRNA in 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) and/or DOPE liposomes [113]. Coadministration of the mRNA for GM-CSF increased OVA-specific cytolytic responses in the same research. Another study found that subcutaneous distribution of LNP-formulated mRNA expressing two melanoma-associated antigens inhibited tumor growth in mice, and that co-delivery of lipopolysaccharide (LPS) in LNPs boosted CTL and anti-tumor activity [114]. A study by Kranz et al. [115] reported that mRNA-lipoplexes encoded with DOTMA/DOPE lipids were able to protect antigen-encoding mRNA against extracellular ribonucleases, which accumulated in the spleen and successfully delivered the mRNA into DCs following systemic treatment, leading in the development of an antigen-specific immune response. A preclinical study in mice injected with antibody-encoding mRNA delivery showed promising response against cancer [116]. Similarly, another study on mice inoculated with luciferase expressing Raji lymphoma cells and treated with mRNA-LNP encoding rituximab revealed diminished tumor growth, underscoring the importance of mRNA coated antibodies as a viable therapeutic option for treatment of cancer [117]. In general, mRNA cancer vaccines have shown to be immunogenic in people, but further improvement of vaccination methods based on fundamental immunological studies will almost certainly be required to gain higher clinical effects.

Polymer-based vectors

Polymeric materials though are less clinically investigated than ionizable lipids, they coat mRNA without the hassles of self-degradation and also promote protein expression. The drawback of polymeric materials however are polydispersity and the clearance of large molecules [91]. To improve the stability of the polymeric platforms, structural modifications such as lipid chains, expansion of branch structures and construction of biodegradation-promoting domains is considered [118]. A polyethyleneimine-polyplex nanoparticle carrying mRNA expressing the influenza virus hemagglutinin and nucleocapsid was employed in a research of mRNA vaccinations. mRNA was successfully transported to dendritic cells, transferred to the cytosol, and translated into proteins in this study, resulting in both humoral and cellular immunological responses [119]. However, because extremely positively charged polyethylene-based formulations attach to negatively charged serum proteins, they are more hazardous; as a result, new cationic polymers,

Table 7
Summary of clinical trials for mRNA vaccine and their results in various cancers.

Interventions	Conditions	Results	NCT Number	Sponsor	Study design
VAC_W_bre1_uID/ IVAC_M_uID	Breast Cancer	iNeST IVAC_M_uID is highly efficient in inducing strong poly-epitopic T-cell responses in patients with TNBC in the post-(neo) adjuvant setting	NCT02316457	BioNTech SE	Phase I
mRNA-2752/Durvalumab	Relapsed/Refractory Solid Tumor Malignancies or Lymphoma/ Triple Negative Breast Cancer, HNSCC, Non-Hodgkin's, Urothelial Cancer, Immune Checkpoint Refractory Melanoma, and NSCLC Lymphoma	Tumor regressions was observed in approximately 50% of patients with head and neck cancer with mRNA 2752 and durvalumab	NCT03739931	ModernaTX, Inc.	Phase I
CV9103	Hormonal Refractory Prostate Cancer	The two-component mRNA vaccine mediates a strong antitumor response against OVA-expressing tumor cells, not only in a prophylactic but also in a therapeutic setting	NCT00831467/ NCT00923312	CureVac AG	Phase I/II
Dendritic cell vaccine	Prostate Cancer	Adjuvant DCV mitigates the time to biochemical progression	NCT01197625	Oslo University Hospital	Phase I/II
CV9104	mCRPC	CV9104 exhibited antigen-specific immune responses post vaccination	NCT01817738	CureVac AG	Phase I/II
Autologous dendritic cell	mCRPC	Adjuvant therapy with autologous dendritic cell vaccine provided longer median PFS and DSS	NCT01446731	Inge Marie Svane	Phase II
mDC and pDC vaccination	mCRPC	Blood-derived CD1c+ myeloid dendritic cells induced functional antigen-specific T cells which in turn is correlated with an improved clinical outcome.	NCT02692976	Radboud University Medical Center	Phase II
BNT112 and cemiplimab	Prostate cancer	BNT112 induces immune and PSA responses in patients with advanced prostate cancer.	NCT04382898	BioNTech SE	Phase I/II
PSA RNA-pulsed dendritic cell vaccine	Prostate cancer	Escalating doses of PSA mRNA-transfected DCs were administered with no evidence of dose-limiting toxicity or adverse effects, including autoimmunity.	NCT00004211	Duke University, National Cancer Institute	Phase I/II
Lipo-MERIT	Melanoma	Lipo-MERIT vaccine is a potent immunotherapy in patients with CPI-experienced melanoma, and induced strong CD4+ and CD8 + T cell immunity against the vaccine antigens	NCT02410733	BioNTech SE	Phase I
Proteasome siRNA and tumor antigen RNA-transfected dendritic cells	Metastatic melanoma	Tumor antigen-loaded DCs provided partial clinical response, exhibited diffuse dermal and soft tissue metastases, had a complete response.	NCT00672542	Scott Pruitt	Phase I
Langerhans-type dendritic cells electroporated with TRP-2 mRNA	Melanoma	TRP2 mRNA-electroporated LC vaccines produced antigen-specific responses especially in terms of cytokine secretion, cytolytic degranulation, and increased TCR clonality leading to clinical outcomes.	NCT01456104	Memorial Sloan Kettering Cancer Center, Rockefeller University	Phase I
IVAC MUTANOME, RBL001/RBL002	Melanoma	60% of the 125 selected neo-epitopes elicited a T-cell response. No severe adverse drug reactions were reported Vaccination with IVAC® MUTANOME was very well tolerated.	NCT02035956	BioNTech RNA Pharmaceuticals GmbH	Phase I
Autologous Dendritic Cells loaded with MAGE-A3, MelanA and Survivin	Stage IV melanoma	Few patients achieved full remission and/ or survived for >10 years, while 2 patients developed asymptomatic sarcoidosis after treatment with autologous dendritic cells	NCT00074230	University Hospital Erlangen	Phase I/II
TriMixDC-MEL	Stage III/IV melanoma	TriMixDC-MEL is tolerable and results in a high rate of durable tumor responses	NCT01676779	Universitair Ziekenhuis Brussel, RIZIV	Phase II
ECI-006	Melanoma	ECI-006 was generally well tolerated and demonstrated immunogenic response	NCT03394937	eTheRNA immunotherapies	Phase I
TriMix-DC	Melanoma	TriMixDC-MEL was safe and produced immunogenic response. Durable antitumor activity was observed across the investigated iv dose levels	NCT01066390	Bart Neyns	Phase I
TriMix-DC and ipilimumab	Stage III/IV melanoma	TriMixDC provided robust CD8 + T-cell responses in melanoma patients, and in patients with a clinical response	NCT01302496	Bart Neyns, Vrije Universiteit Brussel	Phase II
Dendritic cells with or without cisplation	Stage III/IV melanoma	Combination therapy of DC vaccine and cisplatin is safe and produces immune response but the clinical response is similar to DC vaccine monotherapy	NCT02285413	Radboud University Medical Center	Phase II
mRNA with GM-CSF	Malignant melanoma	Direct injection of protamine-protected mRNA is feasible and safe.	NCT00204607	University Hospital Tuebingen	Phase I/II

Table 7 (*continued*)

| Interventions	Conditions	Results	NCT Number	Sponsor	Study design
mRNA-2416	Relapsed/Refractory Solid Tumor Malignancies or Lymphoma,Ovarian Cancer	mRNA-2416 was well-tolerated at all dose levels. Analyses of tumor post-treatment demonstrate increased OX40L protein expression, elevated PD-L1 levels and pro-inflammatory activity.	NCT03323398	ModernaTX, Inc.	Phase I/II
Dendritic vaccine	Breast cancer and malignant melanoma	Treatment with autologous DCs mRNA was feasible and safe and did not alter the percentage of Tregs in patients	NCT00978913	Inge Marie Svane	Phase I
NCI-4650	Melanoma, Colon Cancer, Gastrointestinal Cancer, Genitourinary Cancer, Hepatocellular Cancer	NCI-4650 was found to be safe and elicited mutation-specific T cell responses	NCT03480152	National Cancer Institute	Phase I/II
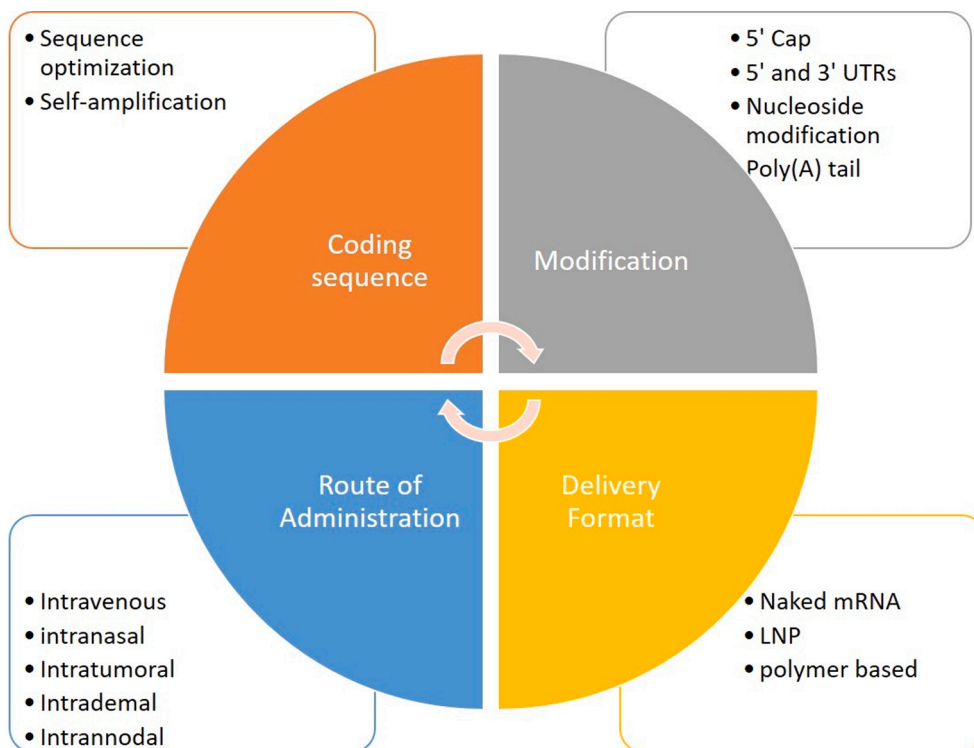
Fig. 3. Key elements that affect mRNA vaccine stability and translation efficacy. LNP – lipid nanoparticles, RNA – ribonucleic acid, UTR – untranslated region.

such as poly(2-dimethylaminoethyl methacrylate) have been created [120]. Polymer-based delivery system research is still in the early stages of development.

Route of delivery

Researchers have investigated various methods for delivery of mRNA vaccines. For instance, mRNA vaccines can be delivered via lipid- or polymer-based system. Dendritic cells can be delivered *ex-vivo* and transferred to the hosts. Targeting of mRNA efficiently into DCs via *in vivo* route remains a major issue. When it comes to solving the delivery problem, there are two key variables to consider: delivery route (the route/portals of entry into the body) and delivery format (stabilized, naked, encapsulated, complexed, adsorbed, etc.). Each delivery route (intradermal, intra tumoral, intranodal, intravenous, subcutaneous, intranasal) has its own set of challenges to overcome, and these challenges will decide the best delivery method. In other words, while developing a vaccine design, the underlying motivation should be a reasonable combination of delivery route and format. Obtaining adequate immunological responses with a certain format and distribution route does not necessarily imply that particular delivery route is

better or it is the best route [4]. In short, the route of administration is significant in determining the efficacy of mRNA vaccine [111].

Both naked and lipid-formulated mRNA administered subcutaneously cause cell transfection, with naked mRNA surpassing lipid-formulated mRNA in terms of translational efficiency. Both formats have demonstrated to induce antigen-specific T cells, but neither has been shown to transfect nodal cells [121,122]. In contrast, a study using lipid nano formulations (approx.70–100 nm) found high and long-lasting translation at the injection site, as well as in CD11c+ cells in draining lymph nodes, leading to delayed tumor growth [114]. Kreiter et al. found that intranodal delivery of adjusted naked antigen-encoding mRNA elicited effective antitumor immunity and mRNA was internalized and translated via micropinocytosis by lymph node resident conventional and cross-presenting CD8a + DCs [121]. Another study by Thielemans et al. validated the potency of intranodal delivery and format in additional tumor models [123]. Thielemans et al. pioneered intertumoral administration to DCs. When in vivo transfected with Tri-Mix, their findings show that naked mRNA is mostly picked up by cross-presenting CD8a + DCs, and that these cells can reawaken T cells at the tumor site as well as move to the draining lymph. The mRNA-encoded secreted proteins might relieve part of the load on immune cells by

lowering MDSC repression, boosting DCs, and activating T cell lysis, which improved tumor growth delay when paired with PD-1 inhibition [123].

Future perspectives and conclusion

With the development and global approval of mRNA vaccines against SARS-CoV-2 virus in the last year have outscored the potential of mRNA technology. Most patients with cancer are non-responsive to current immunotherapies, frequently patients experience relapse and subsequently toxicities to therapies. In this context, therapeutic cancer vaccines is an appealing option to immunotherapy setting because of their potential for safety, specificity, and long-term response due to immunological memory stimulation [21]. The favorable features of potency, fast and relative low-cost production of mRNA vaccine provide an attractive platform for cancer therapy. The mRNA cancer vaccine can be a preferred combination agent with currently available therapies for long-term cancer treatment considering the favorable safety profile observed to date.

Apart from recent progress in the lipid-based delivery system for mRNA vaccines, chimeric antigen receptor (CAR)-T cell immunotherapy is emerging as an encouraging treatment approach for treating malignancies. CAR-T therapy is a personalized form of cell therapy where patient-T cells are genetically engineered to express receptors allowing them to recognize tumor antigens. The adoptive transfer of genetically modified T cells for expressing a CAR have shown encouraging response against hematological tumors. Given this approach, mRNA electroporation has been utilized to generate T cells with CAR expression in preclinical trials [124,125] and subsequently in human trials [126,127]. The preclinical data showed that Descartes-08, an autologous CD8 + CAR T therapy inhibits development of BCMA CAR-specific myeloma and substantially prolongs host survival. Furthermore, an ongoing clinical trial of Descartes-08 reported a favorable therapeutic index with durable responses upon preliminary analysis in patients with relapsed/refractory myeloma (NCT03448978), thus providing a framework for another study using Descartes-11 which is an optimized or humanized version of Descartes-08 in patients with newly diagnosed myeloma patients and having residual disease after induction therapy [128].

Future research should concentrate on deciphering the immunological pathways triggered by different mRNA vaccine platforms and attempt to improve current techniques based on these mechanisms. Utilizing immune-gene therapy with the transfection of autologous mRNA vaccine is another upcoming approach which deserves exploration. For instance, a phase I/II study is currently underway to determine the efficacy and safety of ELI-002, a lipid-conjugated immune-stimulatory oligonucleotide (Amph-CpG-7909) as an adjuvant therapy with a mixture of lipid- conjugated peptide-based antigens (Amph-Peptides) for minimally residual disease in patients with KRAS/neuroblastoma Ras viral oncogene homolog mutated pancreatic cancer or other solid tumors (NCT04853017).

To conclude, despite multifaceted challenges remain in the development of mRNA vaccines, such as extremely large size, susceptibility to enzymatic degradation and instability, the durable efficacy of the RNA in various stages of clinical trials deserves consideration. The work of mRNA on other types of cancer such as ovarian cancer, gastrointestinal cancer remains to be explored. However, delivering mRNA to specific organs, tissues, or cell types remains a significant challenge in the area. It is thus necessary to identify the validated biomarkers that can predict mRNA vaccine efficacy and be utilized for further optimization of the vaccine. Additionally, combination therapies of mRNA with immune checkpoint inhibitors and other immunosuppressing drugs are showing promise [129], nevertheless, more research is needed to determine the most effective combinations and the optimal drug dose for each component. mRNA vaccines will become a significant class of medicine as delivery technologies and vaccine formulations improve, allowing them to effectively combat a variety of health conditions such as

infectious diseases and malignancies.

Author contributions

All authors contributed to manuscript conception, preparation, and approved the final version of the manuscript for submission.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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